Chapter 10

MICROBIAL BIOMASS IN SOIL: MEASUREMENT AND TURNOVER

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I. INTRODUCTION 416
II. MEASUREMENT OF SOIL BIOMASS BY DIRECT MICROSCOPY 417
  A. Principle of the Method 417
  B. Presentation of the Organisms for Microscopy 418
  C. Techniques for the Observation of Soil Organisms 420
  D. Counting and Sizing Procedures 423
  E. Conversion of Biovolume to Biomass 424
III. MEASUREMENT OF SOIL BIOMASS BY ANALYSIS OF SOIL FOR SPECIFIC BIOMASS CONSTITUENTS 426
  A. Principle of the Method 426
  B. Adenosine 5'-triphosphate 426
  C. Muramic Acid 431
  D. Hexosamines Other than Muramic Acid 432
  E. Nucleic Acids 433
  F. Other Biomass Constituents 434

IV. MEASUREMENT OF SOIL BIOMASS FROM THE FLUSH OF DECOMPOSITION CAUSED BY FUMIGATION 434
A. The Flush of Decomposition 434
B. Assumptions Made in Calculating Biomass from the Flush 436
C. Fumigation and Measurement of the Flush 437
D. Measurement of k_c 438
E. The Flush of Mineral Nitrogen Caused by Fumigation 438
V. MEASUREMENT OF BIOMASS FROM THE RESPIRATION RATE WHEN AN EXCESS OF SUBSTRATE IS ADDED 439
VI. CONCORDANCE BETWEEN DIFFERENT METHODS FOR MEASURING MICROBIAL BIOMASS IN SOIL 441
A. Comparisons between Methods 441
B. Discrepancies 443
C. Plate Counts and Microbial Biomass 443
VII. TURNOVER OF MICROBIAL BIOMASS IN SOIL 444
with J. H. Rayner
A. Theoretical Considerations 444
B. Amount of Microbial Biomass in Soil 447
C. Formation and Decay of Microbial Biomass in Soil 447
D. Turnover Time of the Soil Biomass 452
E. Maintenance Energy 454
VIII. CONCLUSIONS 455
REFERENCES 458

I. INTRODUCTION

This chapter is about the size and turnover of the soil microbial biomass, defined as the living part of the soil organic matter, excluding plant roots and soil animals larger than about $5 \times 10^3\ \mu m^3$. For the most part, the biomass is treated as an undifferentiated whole. Methods for distinguishing various sections of the population, such as that devised by Anderson and Domsch (1975) for separating bacterial and fungal respiration, or techniques for distinguishing metabolically active and inactive cells, such as the selective staining procedures of Söderström (1977) or of Macdonald and Spokes (1978), are not considered. We contend that treating the soil biomass in this way, as a single compartment, is useful as a first step in studying the flux of energy and material through the soil population, although recognizing that a full understanding cannot ignore the different roles played by components of the system—the bacteria, the fungi, the algae, and the protozoa, and their interactions with each other, with the larger soil animals, and with plant roots.

II. MEASUREMENT OF SOIL BIOMASS BY DIRECT MICROSCOPY

A. Principle of the Method

The most direct way of estimating soil biomass is to measure the numbers and sizes of organisms in a representative quantity of soil by microscopic observation and then convert the microbial biovolume to microbial biomass or biomass C from the assumed density, water content, and C content of microbial tissue. Technical difficulties are, however, formidable and the results so far obtained (Macdonald, 1974; Jenkinson et al., 1976; Paul and Johnson, 1977) are incomplete and tentative. Counts alone are insufficient for measuring soil biovolume, defined as the volume occupied by living things. Soil organisms span a wide range of sizes, from close to the theoretical minimum size for autonomous existence [set, according to Morowitz (1967), at a diameter of about 0.13 μm, corresponding to a volume of 0.001 μm^3] to the larger worms, say, an adult Lumbricus terrestris with

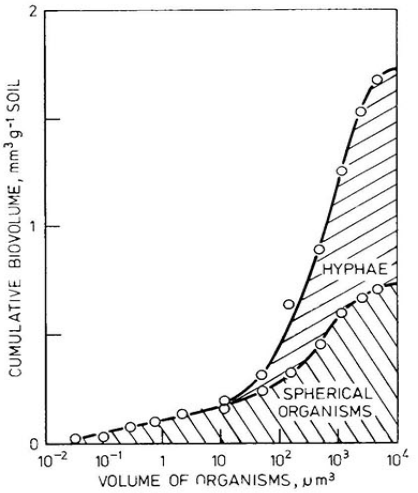

FIG. 1 The contribution made by organisms of different size to the soil biovolume. (Source: Redrawn from Jenkinson et al., 1976.)

a volume of 10^{13} μm^3. Figure 1 shows how soil biovolume is distributed between organisms of different size and shape in an arable soil from Rothamsted. The soil organisms have been grouped into two shapes: "cylindrical," corresponding to fungal and actinomycete hyphae, and "spherical," covering the rest.

Figure 1 also illustrates a curious relationship that holds over a wide range of sizes for the spherical organisms. If the biovolume in this soil is divided logarithmically into equal classes by organism volume, then each class contains the same biovolume (Jenkinson et al., 1976). Thus the class of spherical organisms whose volume limits are 0.1 and 1 μm^3 contains the same biovolume as the 10 to 100 μm^3 class. The numbers of organisms in the two classes are very different, the 0.1 to 1 μm^3 organisms being 100 times more numerous than those in the 10 to 100 μm^3 class. Any procedure that only measures organisms in, say, the bacterial size range will therefore enormously underestimate soil biovolume.

Up to now, all soil biovolume measurements have been truncated and have omitted the very large and the very small. The exclusion of the very large is the most serious. The gap between the minimum size for autonomous existence and the size of the smallest organism that can be seen in the light microscope is relatively narrow compared to the size range of the whole population. Very small organisms, although numerous and perhaps of great ecological importance, are unlikely to contain much biovolume (Jenkinson et al., 1976). Using the electron microscope, Balkwill and Casida (1973) showed that the 0.01 to 0.03 μm^3 class contained fewer organisms than did the 0.03 to 0.1 μm^3 class.

B. Presentation of the Organisms for Microscopy

The most direct way of enumerating and sizing organisms in soil is to examine thin sections. These have been used for measuring the length of mycelium in soil (Alexander and Jackson, 1955; Burges and Nicholas, 1961; Nicholas et al., 1965; Frederick, 1965; Martinez and Ramirez, 1978) and for investigating the distribution of bacteria in soil aggregates (Jones and Griffiths, 1964; Mayfield, 1977). Thin sections are not well suited for biovolume measurements because organisms are often obscured. Nicholas and Parkinson (1967) showed that the total lengths of mycelium in soil from different horizons of a podsol, as measured on soil sections, were only one-tenth to one-third the lengths seen in the corresponding horizons by their version of the Jones and Mollison (1948) procedure.

Because of the limitations of thin sections, most work on the direct microscopic examination of soil organisms has been done by first dispersing the soil and then examining the organisms in thin films prepared from the dispersion. Usually the films are not representative of the whole soil and are concentrated with respect to the microorganisms. The original Jones and Mollison (1948) procedure employed a very gentle trituration procedure

for dispersing the soil. The numbers obtained are less than those obtained by more drastic dispersion procedures. Jenkinson et al. (1976) found that dispersion by trituration gave a count of 3.7×10^9 organisms g^{-1} soil; dispersion by the Waring blender technique (Babiuk and Paul, 1970) gave a count of 5.9×10^9, and dispersion in an ultrasonic cleaning bath in the presence of a detergent gave a count of 5.5×10^9. Anderson and Slinger (1975a) developed a dispersion technique in which an ultrasonicator, normally used to disintegrate organisms, was used at low power for dispersion. Söderström (1979) showed that fungal hyphae were progressively destroyed when a blender was used to disperse the organic horizon of a forest soil. There is probably no satisfactory solution to the problem of dispersing soils for counting and sizing. Treatments drastic enough to dislodge all the organisms from organic debris and to disperse all clumps will disrupt some thin-walled cells beyond recognition, whereas gentler treatments will fail to reveal many organisms.

Three techniques are currently in use for obtaining thin films from soil dispersions for examination under the microscope. In the Jones and Mollison (1948) method, as modified by Thomas et al. (1965), a soil dispersion in molten agar is allowed to settle for a fixed time and a sample then withdrawn at a specified depth so as to exclude coarse inorganic material. The dispersion is allowed to set in a hemocytometer cell and the film, of known thickness, floated off, dried, and mounted for staining. The advantage of this procedure is that the organisms in a given area of film can be accurately related back to the weight of soil dispersed. The disadvantage is that the organisms are embedded in dried agar, which can cause difficulties during the subsequent staining stage.

The soil smear technique is the oldest (Conn, 1918) and is still widely used (Babiuk and Paul, 1970; Trolldenier, 1973). A known volume of dispersed soil is dried over a prescribed area on a glass slide. The thickness of the smear may not be uniform, particularly at the edges (Trolldenier, 1973). This difficulty may be avoided by the ratio method, in which a known number of indigo particles (Thornton and Gray, 1934), or polystyrene-latex beads (Frederick, 1965) is added to the suspension. By measuring the ratio of the number of cells in a field to the number of marker particles in the same field, the amount of soil in the field can be calculated. The ratio technique is useful when organisms of similar sizes are being counted, but may be of less value if a range of organisms of different sizes is under observation. Peterson and Frederick (1979) counted the smaller soil organisms using a magnification of 1940x and beads of diameter 1.3 μm; they suggested that the larger organisms (which often made a dominant contribution to the biovolume in their soils) be counted at lower magnification, using larger beads.

In membrane filtration, dilute soil dispersions are put through filters with pore sizes of about 0.4 μm in diameter and the organisms counted directly on the filter (Coûteaux, 1967; Hanssen et al., 1974; Sundman and Sivelä, 1978). Staining can be before (Faegri et al., 1977) or after filtration

(Paul and Johnson, 1977). This technique promises to be quicker than the agar film method and more precise than soil smears. In membrane filtration preparations (and also in soil smears and agar films; see Anderson and Slinger, 1975a) the number or organisms seen is proportional to the quantity of soil taken only when the film is thin. With thick films, all but the surface organisms are obscured and the count obtained (Faegri et al., 1977) is independent of the volume of soil taken. The problem is analogous to that of counting radioactive disintegrations in solid films where self-absorption by the film is significant.

In any procedure for examining organisms in soil concentrates, care must be taken to see that significant parts of the soil population are not discarded. The coarse fraction of soil dispersions often contains many fungal hyphae (Warcup, 1955)—for reasons that can be appreciated from Clough and Sutton's (1978) pictures of sand grains enmeshed in fungal hyphae.

C. Techniques for the Observation of Soil Organisms

1. Phase Contrast Microscopy

Phase contrast is of little value in counting and sizing small organisms against a background of inorganic soil material. It is, however, useful with larger organisms and has been used to observe "ghost" hyphae that are not stained with phenolic aniline blue (Frankland, 1974). The potential value of the technique is that it can distinguish between lyzed and unlyzed organisms (Frankland, 1975) and it may well become of increasing importance as procedures for separating organisms from the inorganic part of the soil improve (Faegri et al., 1977). Flanagan and Van Cleve (1977) used phase contrast to examine fungal hyphae that had been stained successively with aniline blue and acetoorcein and in this way were able to distinguish cytoplasmic nucleated hyphae (presumably live) from dead anucleate hyphae.

2. Bright Field Observation

Probably the most widely used stain for bright field microscopy is phenolic aniline blue (Jones and Mollison, 1948; Nicholas and Parkinson, 1967; Jenkinson et al., 1976; Frankland et al., 1978; Martinez and Ramirez, 1978). It has the advantage of staining a wide range of soil organisms, although certain bacterial spores and some fungal hyphae are not well stained (Jenkinson et al., 1976). Casida (1971) compared a large number of stains and staining procedures for bright field examination of organisms in soil, among which was phenolic rose Bengal, a useful general-purpose stain for soil work (Peterson and Frederick, 1979).

3. Fluorescence Microscopy

Fluorescence microscopy is based on the emission of light in the visible spectrum from organisms stained with fluorescing dyes and illuminated with light of shorter wavelength. With fluorescent staining, counting and sizing is less tiring, and partially obscured organisms are more easily discerned than with bright field microscopy. A further advantage is that organisms below the optical resolution limit can be counted, as organisms stained with fluorescent dyes are light emitters and will be seen as spots of light, however tiny. However, the fluorescent stains currently available all have the disadvantage that they stain a narrower range of organisms than phenolic aniline blue.

Acridine orange (AO) was the first of the fluorescent stains used in soil work (Strugger, 1948; Trolldenier, 1972). Strugger stated that live organisms, which absorb relatively little of the dye, fluoresce green, whereas dead organisms absorb much dye and fluoresce red. Bucherer (1966) showed that although it was possible to use AO to distinguish living and dead cells with certain gram-negative bacteria, this separation could not be extended to soil. Soils normally contain relatively few organisms that fluoresce red with AO (Aristovskaya and Zykina, 1972; Jenkinson et al., 1976). AO has disadvantages in that success is critically dependent on staining concentration, the dye is strongly adsorbed on nonliving soil constituents (Casida, 1971), and the green fluorescing organisms fade rapidly during counting. Nevertheless it is still widely used (e.g., Zvyagintsev, 1964; Faegri et al., 1977).

Anderson and Westmoreland (1971) introduced a differential fluorescent stain (DFS). This is a combination of two stains: tris[4,4,4-trifluoro-1-(2-thienyl)-1,3 butanediono] europium, which complexes with the nucleic acids of bacterial cells (Scaff et al., 1969), and the fluorescent brightener Na_2 4,4'-bis[4-anilino-6-bis(2-hydroxyethyl)amino-S-triazin-2-ylamino] 2,2'-stilbene disulfonate, which was found by Darken (1962) to be effectively absorbed by growing cultures of bacteria, yeasts, actinomycetes, and higher fungi. Anderson and Westmoreland (1971) found that their combined stain revealed fewer bacteria in Jones-and-Mollison-type soil agar films than did phenolic aniline blue. A similar result was obtained by Jenkinson et al. (1976). However, Anderson and Slinger (1975a, 1975b) improved the procedure for staining with DFS and found that it then gave cell numbers greater than those with phenolic aniline blue. The improved stain is said to differentiate viable (or recently dead) cells, which fluoresce red, and dead cells, which fluoresce green. It is particularly useful in studying the larger cells in soil (Johnen and Drew, 1978).

One of the best fluorescent reagents for soil work is fluorescein isothiocyanate (FITC), which is bound to proteins (Babiuk and Paul, 1970). Using a Rothamsted soil, Jenkinson et al. (1976) found that the number of organisms counted in the bacterial size range (diameter 0.3 to 1.6 μm) with fluorescein isothiocyanate was very similar (4.7×10^9 g^{-1} soil) to the number

counted with phenolic aniline blue (4.9×10^9 g^{-1}). With larger organisms (diameter 7 to 21 μm), phenolic aniline blue gave a count 6 times larger than that with FITC. Many hyphae stained with phenolic aniline blue were not stained with FITC.

Water-soluble aniline blue has recently been introduced as a fluorescent stain for fungal hyphae from soil (Paul and Johnson, 1977) and shown to reveal a greater length of hyphae than phenolic aniline blue under bright field illumination. Water-soluble aniline blue is bound to the polymer β-1,3-glucan (Currier and Strugger, 1956) in fungal cell walls. The magnesium salt of 1-aniline-8-naphthalene sulfonic acid, which fluoresces when bound to proteins, was used by Mayfield (1975, 1977) to stain soil microorganisms in situ. However, Paul and Johnson (1977) found it less useful than FITC.

4. Electron Microscopy

The lower limit of particle size that can be resolved by visible light is about 0.2 μm (Humphries, 1969), and many soil organisms are smaller than this (Nikitin, 1964; Bae et al., 1972). Scanning electron microscopy (Gray, 1967; Hagen et al., 1968; Dart et al., 1969; Todd et al., 1973; Clough and Sutton, 1978) can give useful information on where and how microorganisms are distributed in soil, but the problems of developing it into a quantitative tool for assessing soil biovolume and biomass have not yet been solved.* Scanning electron microscopy has been used to enumerate aquatic bacteria retained on membrane filters (Drier and Thurston, 1978) and it may be possible to extend the technique to soil organisms. Direct scanning electron microscopy suffers from the same disadvantage as other procedures for the direct examination of organisms on exposed soil surfaces: there is no guarantee that numbers and sizes of the visible organisms are a valid guide to numbers and sizes of the obscured organisms. However, the main difficulty arises because of the heterogeneity of soil over the very small areas scanned: an enormous number of fields would have to be examined to give reliable quantitative data.

Transmission electron microscopy has been used primarily for qualitative work (Bae and Casida, 1973; Foster and Rovira, 1973) but Nikitin (1973) and Nikitin and Makarieva (1970) obtained quantitative data for two soils, from which they estimated microbial biomass. Bae et al. (1972) and Balkwill et al. (1975) used transmission electron microscopy to examine the size distribution and morphology of cells in concentrates from soil, but did not determine the soil biovolume.

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*Foster and Martin, Chapter 2 of this volume.

D. Counting and Sizing Procedures

With the light microscope, cells are usually counted within a field delimited by an eyepiece graticule, although counting is sometimes done on photographs (Anderson and Slinger, 1975a). The disadvantage of photography is that organisms just out of focus can easily be missed; when counting on the microscope it is usual to focus up and down on the soil film. Sizing can be done with an eyepiece micrometer, but it is much faster (Jenkinson et al. 1976) to match by eye the area of an organism with the area of a circle of known size on an eyepiece graticule (May, 1965).

Hyphal lengths can be measured by projecting the image of the field and using a tachometer (Thomas et al., 1965; Frankland et al., 1978; Sundman and Sivelä, 1978) or by using a grid graticule in the eyepiece (Jenkinson et al., 1976). The grid graticule can also be used to arrange hyphae into different diameter classes.

In the original Jones and Mollison (1948) procedure, 20 randomly distributed fields were counted on each of four replicated agar films. Trolldenier and Schafer (1972) found that a 10% coefficient of variation could be attained by counting six fields on each of 10 smears, four fields on 13 smears, two fields on 21 smears, or one field on 38 smears. Precision was lost more rapidly by decreasing the number of smears examined than by decreasing the number of fields per smear. Frankland et al. (1978) examined the comparable problem of measuring hyphal lengths in cultures of Mycena galopus growing on birch leaves; again the best strategy was to increase the number of replicate cultures examined, at the expense of the number of films prepared per culture and of the number of fields examined per film. Cassell (1965) developed a rapid graphical method for estimating the number of fields that must be examined in order to reach a given precision.

The relationship illustrated in Fig. 1, that the class of "spherical" organisms with volumes of between 1 and 10 μm^3 contains the same biovolume as the class with volumes between 10 and 100 μm^3, means that the first class will contain 10 times as many organisms as the second class (Jenkinson et al., 1976). To measure biovolume to a given precision with the minimum effort, the same number of organisms must be counted in, for example, the 1 to 10 μm^3 and 10 to 100 μm^3 size classes. As the size of the organisms increases, the area scanned must also increase. Ultimately a point is reached where the whole area of film or smear contains too few organisms for the required precision. At this stage, methods based on the examination of larger quantities of soil must be introduced, for example, Bunt and Tchan's (1955) procedure for the staining and direct counting of soil protozoa or El-Din Sharabi and Pramer's (1973) method for the direct counting of soil algae under the fluorescence microscope.

The principal drawback to direct microscopy as a method for measuring soil biovolume and hence biomass is the labor involved. Successful counting and sizing is somewhat subjective, depending as it does on judgments on the colour, shape, and brightness of organisms that are often partially obscured by organic and inorganic soil debris. Such judgments are difficult to program, and it is unlikely that a fully automated procedure can be developed in the near future.

E. Conversion of Biovolume to Biomass

1. Shrinkage

Organisms shrink and collapse during drying so that the size of an organism on a dry agar film or soil smear will be different from its size in moist soil. Errors introduced when calculating soil biovolume from measurements on dried films can be positive, as when a cylindrical hypha collapses onto the plane of the film and so appears thicker than it was when wet or, more usually, negative, as when organisms shrink in both dimensions in the plane of the film.

These dimensional changes may be avoided by not drying the organisms (as in Babiuk and Paul's 1970 FITC technique). Alternatively, the techniques used in electron microscopy to minimize shrinkage and distortion during the drying of wet tissue (Boyde et al., 1977)—for example, freeze-drying or critical point drying (Cohen, 1977)—could perhaps be applied. A third approach is to measure shrinkage and use the results to convert measurements made on dry tissue to a wet tissue basis (Jenkinson et al., 1976).

Other errors in calculating biovolume from microscopic observation are caused by the capsule-like areas which surround certain microorganisms in soil. These areas are often not stained (Casida, 1971), so that an arbitrary decision must be made as to the true dimensions of an organism. Likewise, the use of stains which stain cell contents rather than cell walls may lead to underestimates of cell size.

2. Conversion of Biovolume to Biomass C

To convert biovolume to biomass C it is necessary to know cell density, cell dry matter content, and the C content of the dry matter. The density of moist microbial tissue varies during the growth of an organism and also from species to species. Values of about 1.1 g cm^{-3} have been found in bacteria and bacterial spores (Ruffilli, 1933). Allison (1924) found the density of algal cells to be close to 1.1, with protozoan cysts having a density of 1.05. The densities of fungal spores ranged from 1.02 to 1.53 g

cm^{-3} (Yarwood, 1952). Recently Faegri et al. (1977) measured the density of bacteria in preparations from soil, as distinct from organisms grown in vitro and found a value of 1.3 g cm^{-3}. This value, however, was obtained after gradient centrifugation using cesium chloride, so that cell dehydration may have occurred, giving a high value for cell density.

Luria (1960) gives the dry matter contents of nine different species of bacteria; the mean was 21%. The dry matter content of soil microarthropods was found to vary from 17 to 25% (Chernova et al., 1971). From an examination of published work, Yeates (1979) suggested a dry matter content of 25% for soil nematodes. The mean dry matter content of fruiting bodies from 15 species of higher fungi was 12.4 ± 6.7% (Hawker, 1950). Fungal spores often contain much less water than this; thus Yarwood (1950) showed that the dry matter contents of airborne spores from nine different fungi varied from 25 to 94%. Van Veen and Paul (1979) cultured a range of bacteria, fungi, and yeasts under different moisture stresses and showed that water content decreased as water stress increased. In general, their organisms, all of which had been isolated from soil, contained more (and sometimes much more) than 20% dry matter, commonly taken as the dry matter content of soil organisms. Casida's (1971) observation, that there are optical differences between organisms as found in soil and as cultured, strongly indicates differences in dry matter content. Most of the recent work suggests that the dry matter content of the soil biomass is greater than 20%, and a value of 25% is provisionally proposed in the absence of measurements of the water content of soil microorganisms in soil.

The C content of dry organisms is much less variable than the contents of the other constituent elements (Bowen, 1966) or indeed than the dry matter content of living organisms. Pinck and Allison (1944) analyzed hyphae from 12 different fungi and found the mean C content to be 47.3%, with a standard deviation of ±3.7%. Anderson and Domsch (1978a) found the mean C content of mycelia from 16 species of soil fungi to be 47.4 ±3.7%; for 12 species of soil bacteria it was 43.6 + 4.6%. Jenkinson (1976) found a mean C content of 46.6 ± 2.3% for a group of 13 organisms that included bacteria, yeasts, fungi, actinomycetes, and earthworms.

If the wet density of soil organisms is taken as 1.1 g cm^{-3}, the dry matter content as 25%, and the C content as 47%, then the factor for converting moist biovolume (in cm^3 g^{-1} soil) to biomass C (in g C g^{-1} soil) is 0.13. If biovolume is measured on dry organisms, it must first be corrected to wet biovolume by allowing for shrinkage. The dry matter content of the soil biomass is much less well established than either the carbon content or the density, and use of a value of 25% could well cause serious errors in calculating biomass from biovolume. Work is needed on the water content of organisms in soil if direct microscopy is to give an accurate measure of biomass.

III. MEASUREMENT OF SOIL BIOMASS BY ANALYSIS OF SOIL FOR SPECIFIC BIOMASS CONSTITUENTS

A. Principle of the Method

The amount of biomass in a soil can, in theory, be gauged from the amount of a particular biomass constituent in that soil. The chosen constituent must meet some stringent requirements. These are (1) that the constituent is present in all parts of the soil biomass in the same (known) concentration at all times; (2) that it is present only in living organisms and not in dead cells or in the other nonliving parts of the soil organic matter; (3) that it can be extracted quantitatively from the soil; and (4) that there is an accurate and precise method for determining it in soil extracts. No cell constituent fully meets these conditions. Several have been tried: adenosine 5'-triphosphate, muramic acid, N-acetylglucosamine, and the nucleic acid bases.

B. Adenosine 5'-Triphosphate

Adenosine 5'-triphosphate (ATP) is a useful indicator of life in soil: it occurs in all living cells and can be estimated accurately and with great sensitivity by the luciferin-luciferase system. Living cells maintain an ATP/ADP concentration ratio that is about 10^8 times the equilibrium ratio. This not only provides the driving force for nearly all the biochemical events in the cell (see Atkinson, 1977) but also ensures that ATP is unlikely to survive long in dead cells or to remain free in the soil. ATP in dead cells is indeed rapidly decomposed (Holm-Hansen and Booth, 1966), as is extracellular ATP in soil (Conklin and Macgregor, 1972; Jenkinson and Oades, 1979). A close link between the ATP content of a soil and the presence of living organisms is shown by Lee, Harris, Williams, Syers, and Armstrong's (1971) finding that irradiation-sterilized soils contained no ATP. The main difficulties in using ATP as a quantitative measure of soil biomass are in extracting it efficiently from soil and in relating the amount of ATP to the amount of biomass.

1. Extraction of ATP from Soil

A successful method of extracting ATP from the soil must (1) release all the ATP from the biomass; (2) inactivate the ATP-hydrolyzing enzymes of the biomass; and (3) hold the released ATP in solution against sorption on the soil colloids. A drastic exposure to ultrasonics was found to be necessary for maximum ATP extraction (Jenkinson and Oades, 1979), and there can be little doubt that much of the soil ATP is present in organisms more difficult to disrupt than are the bacteria usually tested in vitro.

ATP is an extremely labile cell constituent; for example, the adenine nucleotide pool in enteric bacteria turns over 30 to 50 times per generation (Chapman and Atkinson, 1977). A good extraction procedure must therefore disrupt the cell machinery quickly and completely so that neither further synthesis nor degradation of ATP can occur. A measure of this is given by the adenylate energy charge, defined as (ATP + 1/2 ADP)/(ATP + ADP + AMP), where ADP is adenosine diphosphate and AMP is adenosine monophosphate. Living cells commonly have charge values between 0.8 and 0.95 (Chapman and Atkinson, 1977), and values much below this are indicative of enzymic degradation of ATP during extraction. Work on bacteria (Lundin and Thore, 1975a) has shown that reagents containing strongly chaotropic anions such as trichloracetic acid and perchloric acid resulted in extracts with a high energy charge and a complete and irreversible inactivation of nucleotide-converting enzymes. Extracts prepared with butanol, chloroform, formic acid, and sulfuric acid gave lower energy charges. No one has yet measured energy charge in extracts of terrestrial soils, although methods are now available (Holm-Hansen and Karl, 1978). It has, however, been measured in $NaHCO_3$ extracts of certain marine sediments and found to vary between 0.3 and 0.7 (Wiebe and Bancroft, 1975).

ATP, like phosphate, can be held as the anion on positively charged sites in soil over a wide pH range. However, at low pH it can also be sorbed on negative sites as the cation, formed by protonation of the $-NH_2$ group on the adenine part of the molecule (pK_a 4.1). Most of the extractants that have been tried on soil were originally developed for extraction of ATP from microbial cultures in vitro, and it is not surprising that sorption effects often seriously limit their efficiency.

The most widely used acidic extractant is cold 0.3 M H_2SO_4, introduced by Lee, Harris, Williams, Armstrong, and Syers in 1971, and used subsequently by Greaves et al. (1973), Ausmus (1973), Karl and La Rock (1975), Afghan et al. (1977), Holm-Hansen and Karl (1978), Eiland (1979), and Jenkinson and Oades (1979). Lee, Harris, Williams, Armstrong, and Syers (1971) showed that recoveries of added ATP by the H_2SO_4 method varied from 24 to 85% in a way that was not directly related to clay content. However, although not the only factor influencing recovery, clay does have an effect: Ausmus (1973) found that recoveries of added ATP were greater from a synthetic soil made up of sand than from one made up of kaolinite.

Jenkinson and Oades (1979) showed that recoveries of added ATP by 0.5 M trichloracetic acid could be greatly improved by adding a cation (paraquat) and an anion (phosphate) to displace the ATP from the soil colloids. They found that recoveries were generally better in sandy soils than in clays. Organic matter also influences the recovery of added ATP. More ATP was recovered from a soil with 0.8% organic C than from an otherwise similar soil that contained 2.7% organic C (Jenkinson et al., 1979).

None of the other acid extractants tested has shown promise: cold perchloric acid (Lee, Harris, Williams, Armstrong, and Syers, 1971; Ausmus, 1973; Eiland, 1979), cold formic acid (Conklin and Macgregor, 1972), nor acidified dimethylsulphoxide (Ausmus, 1973).

Neutral and basic extractants have the advantage that the $-NH_2$ group on the adenine part of the molecule is not protonated, thus eliminating sorption as the cation. They have the additional advantage of not being consumed by soil carbonate. The near-neutral N-bromosuccinimide-EDTA-arsenate reagent used in the pioneering soil work by MacLeod et al. (1969) was subsequently found to be a poor extractant for soil (Conklin and Macgregor, 1972; Ausmus, 1973). Boiling 0.1 M $NaHCO_3$ was used by Bancroft et al. (1974), Christian et al. (1975), and Hersman and Temple (1978). A modification of this method, in which soil was agitated with cold $NaHCO_3$ and $CHCl_3$ (to lyze cells), was developed by Paul and Johnson (1977) to extract ATP. Recoveries of added ATP were good and soil texture had little effect on extraction efficiency. This modified $NaHCO_3$ method was tested by Jenkinson et al. (1979) and found to give better recoveries of added ATP from soils than the trichloracetic acid-phosphate-paraquat reagent. The amounts of "native" ATP found in the soils were, however, much less by the $CHCl_3$-$NaHCO_3$ method. A similar method using 0.01 M Na_3PO_4-$CHCl_3$ was tested by Afghan et al. (1977) on two lake sediments: the measured ATP contents of the sediments were about 10 times greater than by the cold 0.3 M H_2SO_4 method.

Boiling Tris buffer has been widely tested as an ATP extractant in soil work (Lee, Harris, Williams, Armstrong, and Syers, 1971; Conklin and Macgregor, 1972; Ausmus, 1973; Karl and La Rock, 1975; Eiland, 1979), but the results have been disappointing and most authors (although not all—see Kaczmarkt et al., 1976) have found recoveries of added ATP to be much poorer than by the cold 0.3 M H_2SO_4 method. Procedures in which the soil is shaken with aqueous buffer and a nonmiscible alcohol (usually butanol or a butanol-octanol mixture), have also been used (Conklin and Macgregor, 1972; Anderson and Davies, 1973; Anderson and Drew, 1976; Drew et al., 1978) and found (Ausmus, 1973) to give results comparable with those obtained by the 0.3 M H_2SO_4 reagent. The butanol procedure was, however, shown to be less reliable than acid extractants such as H_2SO_4 or trichloracetic acid as an extractant for bacterial ATP in vitro (Lundin and Thore, 1975a).

None of the proposed methods is entirely successful in meeting all three requirements of a good extractant. The most widely tested is cold H_2SO_4. Whatever the method of extraction, it is essential to correct the measured native ATP content of a soil for recovery of added ATP from the same soil. There is need for a comparative study of the different methods on a range of contrasting soils, much as was done by Lundin and Thore (1975a) for a range of bacteria.

2. Measurement of ATP in Soil Extracts

ATP is usually assayed from the amount of light emitted by the firefly luciferin-luciferase system. The postulated steps of the reaction are

$$\text{E (luciferase)} + LH_2 \text{ (luciferin)} + \text{ATP} \xrightarrow{Mg^{2+}}$$

$$\text{E-}LH_2\text{-AMP} + \text{PP (pyrophosphate)}$$

$$\text{E-}LH_2\text{-AMP} + O_2 \xrightarrow[7.4]{\text{pH}} \text{oxyluciferin} + \text{E} + CO_2 + \text{AMP} + h\nu$$

The procedure is extremely sensitive, and 10^{-14} mol of ATP can be measured (Karl and Holm-Hansen, 1976). Shortly after mixing, the light output reaches a maximum and then slowly dies away: either the maximum intensity attained or an area under the decay curve can be measured (Lundin and Thore, 1975b). Excess luciferin should be present during the assay (Karl and Holm-Hansen, 1976). Light emission can be measured by commercially produced photometers (Karl and Holm-Hansen, 1976; Paul and Johnson, 1977; Stanley, 1976; Picciolo et al., 1978), by liquid scintillation spectrometers (Lee, Harris, Williams, Armstrong, and Syers, 1971; Jenkinson and Oades, 1979; Stanley, 1976), or by photometric and recording apparatus specially developed for ATP work (Ausmus, 1973). The kinetics of the reaction have been studied in detail (Lundin and Thore, 1975b), as have some of the interferences encountered in soil work (Karl and La Rock, 1975). A procedure is available for eliminating interfering ions by sorption of ATP on charcoal and subsequent elution for assay (Hodson et al., 1976), although ATP hydrolysis can occur during charcoal adsorption under certain conditions (Karl and Holm-Hansen, 1978). Care must be taken to measure calibration curves under the same conditions and with the same constituents as the actual soil extracts, because most soil extractants influence the production of light by the enzyme system. ATP can also be estimated by other, less sensitive procedures that may be less subject to interference (Bostick and Ausmus, 1978).

Although the purified luciferin-luciferase system is specific to ATP, there are enzymes present in crude firefly extracts that catalyze the synthesis of ATP from the reaction between ADP and nonadenine nucleotides such as guanosine 5'-triphosphate (GTP). In this way several of the common nucleotides can give light in the assay (Lundin and Thore, 1975b). GTP in particular has been shown to interfere when measuring the ATP content of marine bacteria (Karl, 1978; Karl and Holm-Hansen, 1978); a similar effect has not yet been demonstrated in soil measurements but may well occur. These interferences will be more serious when ATP is measured from the area under the decay curve than from the maximum intensity achieved (Karl, 1978).

3. The ATP Content of the Soil Biomass

If ATP is to serve as a measure of biomass, the ATP content of the soil biomass must be constant and known. Chapman and Atkinson (1977) argued that organisms have evolved sensitive and rapid-acting mechanisms for preserving their adenylate energy charge when the supply of substrate alters. These mechanisms also tend to stabilize ATP concentration, although between wider limits than the energy charge. However, despite these control mechanisms, ATP contents can vary widely under certain circumstances, for example, when growth occurs under conditions of phosphate deficiency (Lee, Harris, Williams, Syers, and Armstrong, 1971; Chapman and Atkinson, 1977; Nannipieri et al., 1978). Phosphate deficiency must be acute to depress the ATP content of the biomass: Jenkinson et al. (1979) showed that the ATP content was little different in soils that had received phosphorus fertilizers regularly and in soils that had not received phosphorus for more than 100 years. Nannipieri et al. (1978) and Oades and Jenkinson (1979) showed that there was a rise in the ATP content of the biomass in glucose-amended soils during and shortly after the period of maximum biological activity.

Two methods have been used to establish the ATP content of the soil biomass. The first is to measure the ATP contents of various organisms in vitro, strike a mean, and assume that this also holds for the soil population. Lee, Harris, Williams, Syers, and Armstrong (1971) originally proposed a value of 3.63 μmol ATP g^{-1} dry biomass, based on work by Strickland et al. (1969) on the C and ATP contents of marine microorganisms. Knowles (1977) listed 40 recent measurements on a wide range of growing prokaryotic organisms and 13 measurements on growing eukaryotic organisms. The mean ATP content of the prokaryotic organisms was 5.0 ± 2.4 μmol g^{-1} dry biomass (omitting one set of values for Nitrobacter) and of the eukaryotic organisms 5.9 ± 1.8.

The second method is to measure ATP in soils of known biomass content. Oades and Jenkinson (1979) and Jenkinson et al. (1979) found a close linear relationship between ATP and biomass (as measured by the fumigation technique; see Sec. IV) that held in a range of 17 contrasting soils developed under different vegetational covers and under different climates. Assuming that all the soil ATP was in the biomass, that the biomass contained 47% C, and that the k constant was 0.45 (Sec. IV. D), then the mean ATP content of the soil biomass in the 17 soils was 6.2 μmol g^{-1} dry biomass, corresponding to an ATP content of 7.2 mg g^{-1} biomass C or to a C/ATP ratio of 138. This value, however, should be used with caution on soils that have recently received additions of substrate or have recently been subjected to treatments such as heating, drying, or freezing.

The close agreement between the mean values for the ATP contents of prokaryotic and eukaryotic organisms grown in vitro (Knowles, 1977) and the measured values for the soil biomass strongly suggests that the con-

centration of ATP in the largely resting soil population is little different from that in actively growing organisms.

C. Muramic Acid

The hexosamine muramic acid (3-0-carboxyethyl-D-glucosamine) is a constituent of the cell walls of bacteria and blue-green algae. It occurs in the N-acetyl form in the polymer murein (also called peptidoglycan, mucopeptide, or glycopeptide), which is the shape-maintaining component of bacteria (Braun and Hantke, 1974). Bacterial cell walls containing muramic acid-based polymers are rapidly degraded in marine environments (Moriarty, 1977; King and White, 1977), but there is as yet no work on how long they persist in terrestrial soils after cell death. As a cell wall polymer, muramic acid is presumably more stable than intermediates of metabolism like ATP and is thus less likely to be affected by soil handling, drying, storage, etc. It has not, however, the ubiquity of ATP, being confined to the prokaryotes (Schleifer and Kandler, 1972).

1. Muramic Acid Content of Prokaryotic Organisms

The cell walls of 5 species of gram-positive bacteria contained 19.4 ± 5.3 mg muramic acid g^{-1} biomass C (Millar and Casida, 1970). Gram-negative organisms, with thinner cell walls, contained less: the mean for 7 species was 7.2 ± 1.3 mg muramic acid g^{-1} biomass C, assuming in both cases that the dry cells contain 47% C. Moriarty (1977, 1978) found similar values: 30 ± 17.2 mg muramic acid g^{-1} biomass C for 7 gram-positive bacteria, some terrestrial and some marine, and 8.1 ± 2.0 mg for 13 gram-negative bacteria, again, some terrestrial and some marine. A blue-green alga contained 11 mg muramic acid g^{-1} biomass C.

2. Muramic Acid in the Soil

Millar and Casida (1970) measured the muramic acid content of a heterogeneous group of 33 soils from different parts of the United States. Taking the muramic acid content of the soil prokaryotes to be 19.4 mg g^{-1} biomass C (see above) and assuming that soil organic matter contains 58% C, a mean of 11.1 ± 6.0% of the organic C in Millar and Casida's soils was in prokaryotic biomass C. This is far greater than even the largest of the figures in Table 2 (Sec. VII. B) for the percentage of total biomass C in soil organic C. If the mean value for the muramic acid content of prokaryotic organisms is less than 19.4 mg g^{-1} biomass C, as it almost certainly is, the biomass calculated from the muramic acid content will be even greater. Unless Millar and Casida's figures for the muramic acid contents of their soils are about an order of magnitude too large, most of the soil muramic acid must be present in nonliving material. It seems

likely, therefore, that muramic acid cannot be used as a measure of microbial biomass or even prokaryotic biomass in terrestrial soils.

D. Hexosamines other than Muramic Acid

Soil hexosamines are comprehensively reviewed in Chap. 5 of this book, and this section is concerned only with their proposed use as a measure of biomass. Chitin, a polymer of N-acetylglucosamine, is a structural constituent of fungal cell walls (Foster and Webber, 1960) that is absent from the tissues of higher plants and of prokaryotic organisms. The amounts of hexosamine in hydrolysates have been used as measures of mycelial biomass during the early stages of wood decay (Swift, 1973a, 1973b) and of leaf litter decay (Frankland et al., 1978).

1. Hexosamine Content of Fungi

The chitin content of fungi varies over a wide range depending on species, nutritional status, and age. Thus the hexosamine content of the fungus Coriolus versicolor, used in Swift's earlier work (1973a), was 2.4 mg hexosamine g^{-1} oven-dry mycelium when grown on wood but 12.4 mg hexosamine g^{-1} oven-dry mycelium when grown in vitro under conditions where N was not limiting. Leaf-grown Mycena mycelium contained 43 mg hexosamine g^{-1} oven-dry mycelium (Frankland et al., 1978); Stereum hirsutum mycelium, growing on wood, 11 mg g^{-1} oven-dry mycelium (Swift, 1978). Götz and Pascher (1962) also found great variation between the hexosamine contents of different fungal species grown in vitro; a Penicillium contained 37 mg g^{-1}, Cladosporium herbarum contained 29 mg g^{-1}, and a Fusarium contained 111 mg g^{-1} dry mycelium.

2. Hexosamines in the Soil

Most surface soils contain 5 to 10% of their total N as amino sugars. Glucosamine is the dominant hexosamine, the only other major hexosamine being galactosamine (Parsons and Tinsley, 1975). Taking the C/N ratio of the hexosamines as 5 and the C/N ratio of surface soils as 10, then 2.5 to 5% of the soil C is in amino sugars. This is greater than current estimates for the percentage of soil C in the whole of the biomass (see, for example, Table 2, Sec. VII. B). Since only a small part of the biomass C is in hexosamines, it is impossible to fit all the soil hexosamine into the biomass. This can be further illustrated by the data in Table 4 (Sec. VIII), where the biomass content of an arable soil from Rothamsted is given as 220 μg biomass C g^{-1} dry soil, based on measurements by the fumigation technique. Greenfield (personal communication) found that 8.7% of the N in this soil was present as hexosamine. This is equivalent to a hexosamine C content of 456 μg g^{-1} dry soil, considerably more than the total biomass C content.

E. Nucleic Acids

Soil hydrolysates contain small amounts of the purine and pyrimidine bases found in deoxyribonucleic acid (DNA) and ribonucleic acid (RNA)—for a review see Anderson (1967). On hydrolysis, DNA gives the purine bases adenine and guanine and the pyrimidines cytosine and thymine, whereas RNA contains the pyrimidine uracil in place of thymine. Both DNA and RNA are extracted from cells by alkali, but RNA is readily degraded in alkaline solution. DNA remains acid-insoluble even after treatment with hot alkali, so that it should be found in the humic acid fraction of an alkaline extract of soil, whereas soluble degradation fragments of RNA should be found in the fulvic fraction (Anderson, 1958).

Nucleic acids added to soil are rapidly broken down (Bowman and Cole, 1978; Greaves and Wilson, 1970; Bremner and Shaw, 1954), although Greaves and Wilson (1970) showed that RNA was relatively resistant to degradation when internally adsorbed on montmorillonite. The instability of nucleic acids outside the cell led to the idea that the nucleic acid content of a soil could serve as a measure of its biomass content.

1. The Nucleic Acid Content of Soil

Anderson (1961) found the total DNA bases in humic acids from a range of Scottish soils to vary from 0.015 to 0.61 μmol bases g^{-1} soil, and suggested (Anderson, 1979) that these soils contained too much DNA for all of it to be in microbial cells. He also obtained other results inconsistent with the hypothesis that all the soil DNA is in the biomass: thus the DNA base content of one soil increased down the profile, although the percentage of total C (and presumably biomass) decreased. Further evidence came from the proportions of the four bases in soil hydrolysates. Although Anderson's preparations should have contained only DNA, the Chargaff rule for DNA, that adenine and thymine are present in equimolar proportions, as are guanine and cytosine, was only obeyed in 1 soil out of 13. This suggests that the DNA from soil was not unaltered material from living cells but a degradation product that had undergone considerable alteration.

The total nucleic acid content of bacteria can be as high as 15% with two or three times more RNA than DNA (Vendrely, 1946; Kihlberg, 1972). Recently RNA and DNA bases were measured in hydrolysates of podsolic forest soils from Russia by Aseeva et al. (1977), who found that roughly equal amounts of RNA and DNA were present. Their results strongly suggest that there was too much DNA in the soil for all of it to be in the fungal and bacterial biomass, as determined by direct microscopy. Taking all their soils and soil horizons (including litter layers) together, they found a rough linear relationship between biomass, as measured by direct microscopy, and DNA, given by the equation

$$\text{DNA} = 0.2(\text{biomass})$$

where DNA and biomass are expressed in the same mass units. Thus, if all this DNA was in the biomass it would contain about 20% DNA. Luria (1960) gives the DNA content of bacteria as 3 to 4% of the dry weight, and other soil organisms are unlikely to contain more.

Lid Torsvik and Goksöyr (1978) showed that bacteria isolated from an organic soil by fractional centrifugation contained 8.4×10^{-15} g DNA per cell. This is compatible with established values for the DNA content of bacteria, and they suggested that all the DNA in this fraction was present in live or very recently dead bacteria. Thus, although the DNA in isolated soil organisms may serve as a measure of the amount of biomass isolated, the work done to date on DNA and RNA in soil suggests that the prospects for using the nucleic acid content of a soil as a measure of its biomass content are not good.

F. Other Biomass Constituents

Few cell components have yet been tested as biomass indicators in soil. Among those investigated in the simpler aquatic environment are poly-β-hydroxybutyrate (Herron et al., 1978) and the lipopolysaccharide (LPS) found in the cell walls of gram-negative bacteria (Watson et al., 1977). Poly-β-hydroxybutyrate is an endogenous cell storage polymer known from electron microscopic evidence to be present in certain bacteria in soil (Chap. 2). It has been measured in estuarine muds (Herron et al., 1978) and may have potential as a measure of prokaryote biomass in terrestrial soils. An elaborate purification procedure was necessary before measurement by spectrophotometric assay (Law and Slepecky, 1961), and terrestrial soils may contain too much other chloroform-extractable material for a successful assay. Nothing is yet known about its persistence outside the cell in terrestrial environments. Diaminopimelic acid (DAP) is a constituent of prokaryote cell walls that has been used as a measure of bacterial growth in the rumen (El-Shazly and Hungate, 1966). It has been proposed as a measure of bacterial biomass in soil (Steubing, 1970), but insufficient work has yet been done to assess its value.

IV. MEASUREMENT OF SOIL BIOMASS FROM THE FLUSH OF DECOMPOSITION CAUSED BY FUMIGATION

A. The Flush of Decomposition

When a soil is exposed to a volatile fumigant, the fumigant removed, and the soil incubated, respiration is often initially less than in an unfumigated control. After some hours, the respiration rate of the fumigated soil

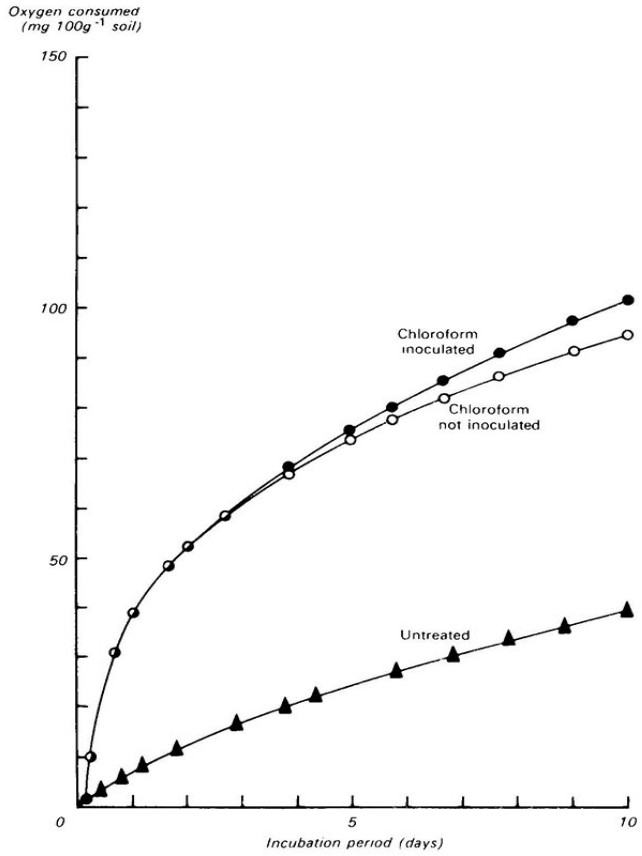


FIG. 2 Effect of $CHCl_3$ fumigation, with and without inoculation, on O_2 consumption by soil. (Source: Jenkinson and Powlson, 1976a.)

becomes much greater than that of the control but subsides again within a few days (Fig. 2). Thus, over a short period, fumigated soil consumes more oxygen (Darbyshire and Russell, 1907; Russell and Hutchinson, 1909; Birch, 1959; Jenkinson and Powlson, 1976a) and evolves more CO_2 (Waksman and Starkey, 1923b; Powlson and Jenkinson, 1976) than untreated soil. Fumigation usually causes an immediate increase in the extractable NH_4^+ content of soil (Waksman and Starkey, 1923a; Russell and Hutchinson, 1909); still more NH_4^+ is released when the soil is subsequently incubated (Russell and Hutchinson, 1909; Waksman and Starkey, 1923a; Powlson and Jenkinson, 1976).

Fumigation thus briefly accelerates the decomposition of soil organic matter. This "flush of decomposition" is defined as the amount of CO_2 evolved (or O_2 consumed or N mineralized) by a fumigated soil when incubated for a given time, less the CO_2 evolved (or O_2 consumed or N mineralized) by the same amount of untreated soil in the same time. Jenkinson (1966), reviving an earlier suggestion (Störmer, 1908), proposed that this flush was due to the decomposition of organisms killed during fumigation by the survivors or, if an inoculum had been added, by the introduced population. The size of the flush can then be related to the size of the biomass by the expression

$$B = F/k_c$$

where B is soil biomass C, in μg C g^{-1} soil; F is the CO_2-C evolved by fumigated soil during incubation, less that evolved by unfumigated soil incubated for the same time under the same conditions, also in μg C g^{-1}; and k_c is the fraction of the biomass C mineralized to CO_2 during incubation.

B. Assumptions Made in Calculating Biomass from the Flush

Jenkinson (1966, 1976) has summarized the assumptions made in calculating biomass from the flush of CO_2 evolved by a soil that has been fumigated and then incubated. These are:

1. The C in dead organisms is mineralized to CO_2 more rapidly than that in living organisms, i.e., the protected substrate that is a living cell becomes available to others on its death.
2. The kill is substantially complete.
3. The biomass dying in the unfumigated control soil during incubation is negligible compared to that killed by fumigation.
4. The fraction of the killed biomass C mineralized (k_c) is the same in different soils. Two further assumptions are implicit in this. The first is that a given mix of killed organisms will decompose to an

equal extent in different soils. The second is more fundamental, that a single value of k_C can be applied to soils which may differ quantitatively and qualitatively in their biomasses.

5. That fumigation has no effect on the soil other than the killing of biomass—the key assumption. This implies that microbial metabolites not in the biomass are not altered, chemically or physically, during fumigation to render them decomposable during the flush. Although it is difficult to exclude such mechanisms entirely, there is evidence to suggest that they make, at most, a small contribution to the flush. The decomposability of dead organisms themselves is little altered by a subsequent fumigation (Jenkinson, 1976; Anderson and Domsch, 1978a) so that any metabolites contributing to the flush would have to exist outside the biomass and be different from those inside it. Anderson and Domsch (1978a) also showed that chloroform fumigation did not alter the evolution of CO_2 from soil that contained heat-killed fungi and their exocellular metabolic products.

   However, the strongest argument against a significant contribution to the flush from altered microbial metabolites comes from work showing that about the same amount of CO_2, of about the same specific activity, was evolved from soil nonuniformly labeled with ^{14}C, whether treated with chloroform or exposed to radiation (Jenkinson, 1966). This strongly suggests that both treatments operate mainly on the same fraction of the soil organic matter. A metabolite would have to have some remarkable properties to be rendered decomposable to about the same extent by treatments as different as irradiation and exposure to chloroform vapor.

C. Fumigation and Measurement of the Flush

The fumigant must give a near-complete kill and then be completely removed before incubation commences. Chloroform (Birch, 1959; Jenkinson, 1966), methyl bromide (Powlson and Jenkinson, 1976), or carbon disulfide (Kudeyarov and Jenkinson, 1976) have been used, but of these chloroform is most convenient. Shields et al. (1974) showed that chloroform fumigation reduced the bacterial plate count of a cultivated brown chernozemic soil by 99.9%, the soil immediately after fumigation containing about 2×10^5 organisms g^{-1}. Enough organisms survive chloroform treatment for an inoculum not to be essential: with the more lethal methyl bromide an inoculum must be used. Incubation must be done aerobically under prescribed conditions of temperature and water content; if these are changed, k_C must be redetermined.

Serious errors in measuring the flush occur in calcareous soils, particularly if they contain little biomass (Jenkinson and Powlson, 1976b). Errors are also likely in soils that have recently received large additions

of substrate because of the large and rapidly changing evolution of CO_2 from the unfumigated control soil, against which the flush must be measured.

D. Measurement of k_c

No measurements of k_c have yet been made on the soil population in situ. Present values have been obtained by incorporating organisms grown in vitro into soil, fumigating, and measuring the proportion of organism C mineralized under the standard incubation conditions. For the 13 organisms used by Jenkinson (1976), the mean value of k_c (measured at 25°C in one soil) was 0.50 ± 0.08. For the 27 species of fungi and bacteria examined by Anderson and Domsch (1978a) the weighted mean (using a 1:3 ratio for bacterial to fungal biomass) was 0.41, measured at 22°C in four soils. A value of 0.45 for k_c at 25°C fits the published results reasonably well.

Two factors operate that make it likely that a single value of k_c may be applied to different soils without serious error. One is that the values of k_c for different organisms, although by no means all the same, do not span a very wide range (Anderson and Domsch, 1978a). The other is that, for soils developed under natural conditions, there are similarities between different soils in the pattern of distribution of different types of organisms, although the amounts may differ widely (Jenkinson et al., 1976).

E. The Flush of Mineral Nitrogen Caused by Fumigation

The ratio

$$\frac{\text{N mineralized by fumigated soil during incubation}}{\text{N mineralized by unfumigated soil during incubation}}$$

is greater than the corresponding ratio for either CO_2 evolved or O_2 consumed (Ayanaba et al., 1976). The flush of mineralization should therefore be the most sensitive measure of biomass. However, the N content of the soil population is not known accurately, in contrast to the situation with C. The C content of nearly all organisms falls inside the range $47 \pm 10\%$ (on a dry matter basis), whereas the N content is much more variable. For example, the C/N ratio of fungal hyphae is often in the 10 to 12 range, that of the bacteria usually between 3 and 5 (Jenkinson, 1976). Thus the k factors for the different sections of the soil population are likely to cover a much wider range with N than with C. As an extreme example, Jenkinson (1976) found that in six different species of bacteria, the nitrogen k factor (k_n) varied from 0.45 to 0.59, whereas in two species of fungi N was actually immobilized, giving k_n values of 0. Amato and Ladd (unpublished)

also found that k_n was less for fungi than for bacteria, although the difference was less marked. Thus k_n for a Pseudomonas sp. was 0.48, for Bacillus sp. 0.43, for Rhizoctonia sp. 0.23, and for Penicillium sp. 0.28. The k_n factor is therefore much more sensitive to change in the proportion of bacteria to fungi than the k_c factor, making it difficult to strike a single value for k_n that is valid for a wide range of soils.

Nevertheless, despite these reservations, there is an empirical relationship between biomass C and flush of N(Fn) given by

$$\text{Biomass C} = 8F_n$$

that holds for a wide range of soils, tropical and temperate, cultivated and uncultivated, fertilized and unfertilized (Ayanaba et al., 1976; Jenkinson et al., 1979; Amato and Ladd (1980). It may prove useful as a rough measure of the amount of biomass, but should not be used in soils containing large amounts of decomposable material of wide C/N ratio, in which immobilization of N will affect the measurement of the flush. If biomass C is calculated using a k_c factor of 0.45, rather than 0.5, the equation becomes $B = 9F_n$.

The relationship $B = 8F_n$ suggests that a C/N ratio of 4 holds for that part of the biomass N decomposed during the 10-day incubation period but not necessarily for the whole of the biomass. As pointed out by Ladd et al. (1977), the chloroform-labile biomass N may be more closely related to the cytoplasmic constituents of the soil biomass than to the cell wall N.

V. MEASUREMENT OF BIOMASS FROM THE RESPIRATION RATE WHEN AN EXCESS OF SUBSTRATE IS ADDED

A rapid method for measuring biomass has recently been developed by Anderson and Domsch (1978b). When increasing concentrations of substrate are added to soil the initial respiration rate increases to a maximum value (Fig. 3). The value attained is different in different soils, and Anderson and Domsch showed that it bore a close ($r = 0.96$) linear relationship to the initial biomass content of the soil, as measured by the fumigation method. As incubation proceeds, cells proliferate and the respiration rate then reflects the activities of the new population as well as those of the initial population (Drobnik, 1960).

Of the substrates tested by Anderson and Domsch (1978b), glucose was best, presumably because it could be metabolized by the largest proportion of aerobic soil microorganisms under the particular incubation conditions used. The concentration of glucose necessary to give the maximum initial rate of respiration must first be determined for each soil, and the need to do this is a disadvantage of the method as it now stands.

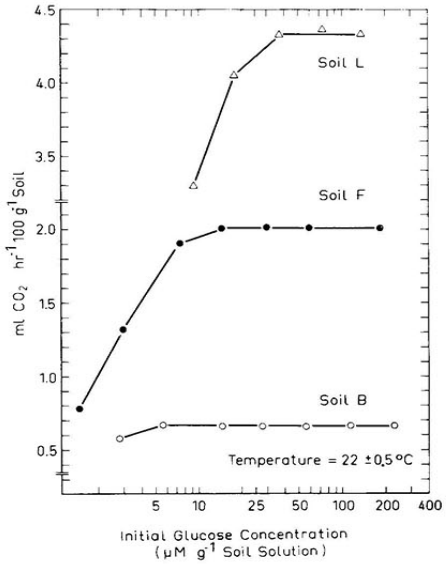


FIG. 3 Response of the biomass in three different soils to different concentrations of glucose. Rate measurements were made 1 hr after glucose additions in soils B and F, 3 hr after addition in soil L. (Source: Redrawn from Anderson and Domsch, 1978b, with permission.)

The fundamental assumption is that the initial respiration rate in the glucose-amended soil is the same for different sections of the population. This requirement can be relaxed if the distribution of biomass between the different sections can be taken as the same in different soils, even though the absolute amounts of biomass differ between soils. Another assumption is that biomass measurements by the fumigation method, against which the initial respiration rates are calibrated, are correct. Difficulties arise because of the shape of the respiration curves in different soils. Some glucose-amended soils respire at a steady rate for several hours before the rate rises as a result of the activity of the newly synthesized population, in others the rate falls initially and then rises, whereas in a third group of soils the

rate increases steadily from the beginning. Anderson and Domsch (1978b) used the respiration rate measured 1 hr after glucose addition—except in soils showing an initial fall, for which they used the minimum rate. As in the fumigation method, the incubation has to be done under the exact conditions used by Anderson and Domsch if their original calibration is to apply.

VI. CONCORDANCE BETWEEN DIFFERENT METHODS FOR MEASURING MICROBIAL BIOMASS IN SOIL

A. Comparisons between Methods

At present, four methods (direct counting, ATP content, the fumigation method, and Anderson and Domsch's CO_2 evolution method) are available for measuring biomass in terrestrial soils, although much work remains to be done to specify the exact conditions under which an individual method may or may not be used. No one has yet compared all four at the same time on a range of soils sufficiently diverse to provide a searching test, although a few comparisons have been made between various combinations.

The fumigation method of measuring biomass was compared with the direct biovolume method in two soils from Nigeria and six from England by Jenkinson et al. (1976). Table 1 shows that, with the exception of an acid woodland soil of pH 3.9, there was close agreement between the two methods. To some extent this agreement must be coincidental, since the direct biovolume measurements include dead cells but exclude organisms outside the diameter range 0.3 to 21 μm. Table 1 also shows four ATP measurements made on the same soils (but not sampled at the same time). There was rough agreement between all three methods of measuring biomass in three of the soils but not in the acid woodland soil.

Nannipieri compared fumigation, direct counting, and the ATP method, using a soil low in organic matter to which substrate had recently been added (Table 1, soils 9 to 12). Although all three methods gave results of the same order of magnitude, there were serious discrepancies, particularly for the ATP method, which was greatly influenced by the amounts of P available to the population during growth.

Nannipieri et al. (1978) measured both microbial biomass (by direct counting, fungi and bacteria being measured separately) and ATP in a soil incubated with glucose. The ATP content of the biomass increased sharply with addition of glucose, particularly when N and P were also supplied, and then fell away again. The peak rate of CO_2 evolution occurred before either biomass or ATP reached its maximum. Similarly Witkamp (1973), working with a fungal culture in sand, found that CO_2 production reached its peak before cell weight and that the ATP content peaked rather sooner than cell weight.

TABLE 1 Estimates of Soil Microbial Biomass by Different Methods

Soil No.	Use and Location of Soil, or Experimental Treatment	Biomass (μg C g^{-1} soil) From Chloroform Fumigation[a]	From Direct Counts	From ATP Content
1	Continuous wheat, receiving farmyard manure annually, England[b]	560	650[e]	430[g]
2	Continuous wheat, no manure, England[b]	220	220[e]	170[g]
3	Calcareous deciduous woodland, England[b]	1230	1820[e]	1040[g]
4	Mixed arable cropping, England[b]	360	470[e]	-
5	Old grassland, England[b]	3710	3780[e]	-
6	Acid deciduous woodland, England[b]	50	390[e]	470[g]
7	Secondary rain forest, Nigeria[b]	540	510[e]	-
8	Arable cropping, Nigeria[b]	280	310[e]	-
9	Parent material[c], low P[d]	330	200[f]	400[h]
10	Parent material[c], high P[d]	180	170[f]	510[h]
11	Parent material[c] with clay, low P[d]	250	190[f]	180[h]
12	Parent material[c] with clay, high P[d]	190	190[f]	240[h]

[a]Using $k_c = 0.45$.
[b]Jenkinson et al. (1976).
[c]Parent material from a sandy loam soil, pretreated with H_2O_2 to remove organic matter and then incubated with 0.1% glucose for 122 hr, with and without additions of clay and phosphate, before biomass measurements were made.
[d]Nannipieri, quoted in Paul and Van Veen (1978).
[e]Assuming that the biomass has a specific gravity of 1.1 and contains 25% dry matter, of which 47% is C.
[f]Corrected for spherical organisms >3 μm diameter that were not counted.
[g]Jenkinson et al. (1979), using a biomass C/ATP ratio of 138.
[h]Using a biomass C/ATP ratio of 250.

B. Discrepancies

There are three conflicting strands of evidence concerning the biomass of soil that has recently been fumigated. When a soil is fumigated, the fumigant removed, and the soil incubated for a few days, there is a large increase in the number of bacteria as measured by plate counting (Waksman and Starkey, 1923a; 1923b; Russell and Hutchinson, 1909; Ridge, 1976). Direct counts, however, show that the biomass in fumigated, incubated soil is a little less than that in the soil before fumigation (Jenkinson et al., 1976). In contrast, the fumigation method of measuring biomass indicates that the new population that developed after fumigation was only 15 to 20% of that originally present (Oades and Jenkinson, 1979). ATP measurements on fumigated, incubated soils were in line with biomass measurements by the fumigation method (Oades and Jenkinson, 1979). This suggests that many of the organisms seen in direct counts of recently fumigated soils are dead and devoid of ATP and also that the rapidly growing bacteria revealed by plate counts in fumigated, incubated soil make up a very small proportion of the biomass of normal unfumigated soil. Plate counts are thus a very bad guide to soil biomass.

There are clear indications that the fumigation method is unsuitable for soils with pH values below about 4.5. Anderson and Domsch (1978a) found that although k_c was very similar in soils of pH 7.5, 5.5, and 5.4, it was significantly smaller in a soil of pH 4.2. The results in Table 1 for soil 6 support this. In this soil the ATP content of the biomass was impossibly large if calculated on the basis of the fumigation method, but of the right order if calculated on the basis of direct biovolume measurement (Jenkinson et al., 1979). These results suggest that a value of k_c much less than 0.5 should be used with strongly acid soils.

It is unwise to rely on a single method for measuring biomass, particularly in strongly acid soils or in situations where the soil population is undergoing rapid changes.

C. Plate Counts and Microbial Biomass

Lee, Harris, Williams, Syers, and Armstrong (1971) showed that bacterial ATP, calculated from plate count data, could only account for a small part of the ATP in the sediments they examined. However, when sterilized sediment (which contained no ATP) was amended with nutrient broth and inoculated with Aerobacter aerogenes, the total ATP content of the sediment was then compatible with the quantity of ATP that would be contained in the bacterial population as enumerated by plate counting. The most probable reason for this discrepancy is that most of the ATP in the unsterilized sediments was present in nonbacterial biomass. Greaves et al. (1973) measured bacterial numbers in peat by direct counting (using FITC) and by plate

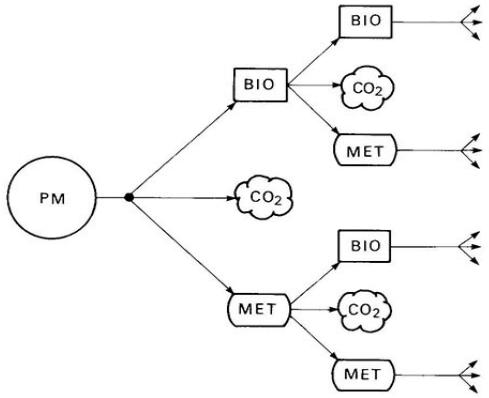
counting. In the particular peat they used, the microbial population was almost exclusively bacterial. The ATP content per cell was impossibly large when calculated on the basis of plate count numbers, but of the correct magnitude when calculated on the basis of direct counts. A similar conclusion was reached by Kaczmarek et al. (1976), who compared ATP contents, direct counts, and plate counts in a range of mineral soils.

The huge discrepancy in soil microbial numbers as determined by plate counts and by direct counts (Skinner et al., 1952; for a review see Casida, 1968) has been explained by postulating that most of the direct-counted organisms are dead. The rough concordance between biomass as measured in natural topsoils by fumigation, ATP content, and biovolume (Table 1: soils 1 to 8) is a strong indication that this explanation is not valid.

VII. TURNOVER OF MICROBIAL BIOMASS IN SOIL
(with J. H. Rayner, Rothamsted Experimental Station)

A. Theoretical Considerations

Several of the models proposed for the turnover of organic matter in soil (McGill, 1972; Hunt, 1977; Jenkinson and Rayner, 1977) incorporate submodels for the turnover of microbial biomass. The model for the soil biomass set out in Fig. 4 incorporates ideas from all of them, although simpler than any in that only three compartments are postulated. In this model an annual input of fresh plant material (A kg C ha^{-1} yr^{-1}) maintains a compartment of undecomposed plant material (P kg C ha^{-1}) in a given layer of soil. The standing crop of biomass is also treated as a single compartment; in this layer it is B kg C ha^{-1}. The stabilized microbial metabolites formed during the decomposition process make up another single compartment containing M kg C ha^{-1}. To handle the model with the limited data currently available, it is necessary to make the further simplification that biomass, stabilized microbial metabolites, and CO_2 are all formed in the same proportion (although not necessarily at the same rate), whether plant material, biomass, or microbial metabolites are undergoing decomposition. The rate constants [i.e., the amount of C leaving a compartment in unit time (1 yr) per unit C in that compartment] are given by r_p for the plant material compartment, r_b for the biomass compartment, and r_m for the metabolite compartment. The fraction of the output from any compartment going to biomass is f_b (i.e., the yield coefficient), to metabolites is f_m, and to CO_2 is $(1 - f_b - f_m)$.



Scheme for the decomposition of plant material (PM) in soil, leading to the formation of microbial biomass (BIO), microbial metabolites (MET), and CO_2

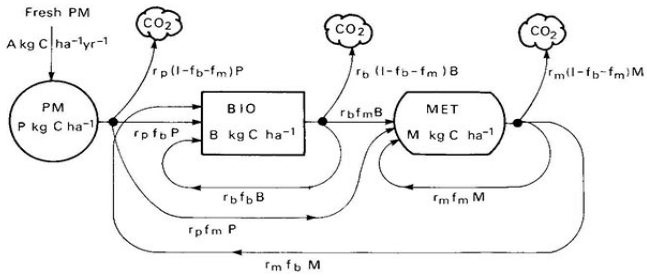


FIG. 4 Decomposition scheme and three-compartment model for the turnover of microbial biomass in soil.

For this model

$$\frac{dB}{dt} = r_p f_b P + r_b f_b B + r_m f_b M - r_b f_m B - r_b f_b B - r_b(1 - f_b - f_m)B$$

$$= r_p f_b P + r_b f_b B + r_m f_b M - r_b B$$

$$\frac{dP}{dt} = A - r_p f_b P - r_p f_m P - r_p(1 - f_b - f_m)P$$

$$= A - r_p P$$

$$\frac{dM}{dt} = r_p f_m P + r_b f_m B + r_m f_m M - r_m f_b M - r_m f_m M - r_m(1 - f_b - f_m)M$$

$$= r_p f_m P + r_b f_m B + r_m f_m M - r_m M$$

Under steady-state conditions

$$\frac{dB}{dt} = \frac{dP}{dt} = \frac{dM}{dt} = O$$

By rearranging the three steady state equations it can be shown that

$$A = \frac{r_b}{f_b}(1 - f_b - f_m)B$$

The turnover time (T) of the biomass under steady state conditions is given by

$$T = \frac{B}{r_b f_b B + r_b f_m B + r_b(1 - f_b - f_m)B}$$

$$= \frac{1}{r_b}$$

For a homogeneous steady state population, such as that in this model,

$$B_t = B_i e^{-r_b t}$$

where B_i is the biomass at a given instant and B_t is the residue of B_i still remaining in the soil after time t has elapsed, ignoring organisms formed during t. The half-life $t_{1/2}$ is then given by

$$t_{1/2} = \frac{\ln 2}{r_b} = T \ln 2$$

Under steady state conditions $t_{1/2}$, the time for the population present at a given moment to fall to half its value is equal to the doubling time (t_D) of the population, so that

$$t_D = T \ln 2$$

The most critical test of this type of model is to follow the movement of a pulse of labeled substrate through the various compartments—see Atkins (1969) for an examination of the mathematics of pulsed tracer flow through multicompartment systems. Steady state models such as the above can also be tested by altering the input and observing the time taken for the various compartments to reach a new steady state.

B. Amount of Microbial Biomass in Soil

Clark and Paul (1970) brought together many of the older soil biomass estimates, and others are given by Barber and Lynch (1977). These estimates are mostly based on various combinations of direct and plate count numbers, converted to biomass by assuming standard sizes for different members of the soil population. As an example, Clark and Paul (1970) estimated that the microbial biomass in the top 30 cm of the Matador grassland site in Saskatchewan contained about 1000 kg C ha^{-1}, with the fungi contributing about twice as much C as the bacteria and actinomycetes combined.

Measurements made by the fumigation method are, in general, larger than the earlier estimates. Some representative fumigation measurements are shown in Table 2; the references in the footnotes contain additional results. With a few exceptions, about 2 to 3% of the organic C in the soil was present in microbial biomass. Of the soils in Table 2, no. 7, a semiarid sandy soil from Australia, developed under native vegetation, contained least biomass and the smallest percentage of biomass C in the organic matter; improved management of the site (soil 8) increased both. Exactly the opposite occurred when a soil under bush regrowth (soil 5) from the humid zone of Nigeria was cleared and cultivated (soil 6); both biomass and the proportion of biomass decreased. In general, situations favoring accumulation of organic matter increase both the amount of biomass and the proportion in the soil organic matter.

C. Formation and Decay of Microbial Biomass in Soil

Energy considerations make it unlikely that more than about 60% of substrate C can be converted to cell C by substrate-limited organisms growing exponentially under aerobic conditions (Payne, 1970; Paul and McLaren, 1975; Payne and Wiebe, 1978). It is not known if conversion efficiencies as high as this are ever attained in soil, and the data reviewed by Wagner (1975) suggest that the figures are much lower.

TABLE 2 Total Organic C and Biomass C in Various Soils

Soil No.[a]	Location	Land Use	Sampling Depth (cm)	Organic C (tonnes ha^{-1})	Biomass C[b] (kg ha^{-1})	Biomass C (as % total organic C)
1(5)	England[c]	Arable	0-23	29	660	2.2
2(7)	England[c]	Deciduous woodland	0-23	65	2180	3.4
3(9)	England[c]	Permanent grassland	0-23	70	2240	3.2
4(20)	Nigeria[d]	Secondary rain forest	0-15	19	760	4.0
5(7)	Nigeria[d]	Bush regrowth	0-17	27	700	2.6
6(10)	Nigeria[d]	Bush regrowth cultivated to maize 2 yr	0-16	22	370	1.7

7(1)	Australia[e]	Unimproved scrub	0-15[g]	21	170	0.8
8(2)	Australia[e]	Improved pasture	0-15[g]	35	430	1.2
9(8)	Australia[e]	Pasture	0-15[g]	39	1170	3.0
10(C)	Germany[f]	Arable	0-10[h]	14	300	2.2
11(F)	Germany[f]	Arable	0-10[h]	28	910	3.2
12(I)	Germany[f]	Arable	0-10[h]	32	620	1.9

[a]Numbers (or letters) in parentheses refer to soil code in original papers.
[b]Using $k_c = 0.45$.
[c]Jenkinson and Powlson (1976b).
[d]Ayanaba et al. (1976).
[e]Oades and Jenkinson (1979).
[f]Anderson and Domsch (1978a).
[g]Assuming weight of 0-to-15-cm layer to be 1750 tonnes ha^{-1}.
[h]Assuming weight of 0-to-10-cm layer to be 1100 tonnes ha^{-1}.

When a simple substrate such as glucose decomposes in soil, microbial biomass is rapidly synthesized during the original attack on the substrate, which is completely consumed within a day or two (Ladd and Paul, 1973). This initial population then serves as substrate for succeeding populations, as do the various microbial metabolites released throughout the decomposition process. With a substrate like plant material the situation is even more complex: the initial attack proceeds at differing conversion efficiencies and at differing rates as the various constituents—the proteins, lignins, carbohydrates, etc.—are attacked by different sections of the soil population.

The new methods of measuring microbial biomass in soil make it possible to determine the overall amount of microbial tissue present at a given time during the decomposition of a given amount of uniformly labeled substrate. Some measurements on simple and on complex substrates are set out in Table 3. With glucose, the largest amount of substrate C present as biomass C was 20%, far less than the theoretical maximum of about 60%. To some extent this is a reflection of the time of sampling, the earliest measurement being made 7 weeks after the addition of glucose, long after the peak production rate of CO_2 and of biomass (Ladd and Paul, 1973; Behera and Wagner, 1974; Nannipieri et al., 1978). With whole plant material, Amato and Ladd's (1980) results show that the peak biomass content occurred much later, about 9 weeks after the addition of labeled medic (Medicago littoralis), a result in accord with the theoretical model for the decomposition of a complex substrate set out by Paul and van Veen (1978). About 10% of the original substrate C was in the biomass at the time of the peak.

The two experiments reported in Table 3 on the decomposition of labeled medic in the field indicate that, other things being equal, biomass declines more rapidly in a sandy soil than in a clay soil. The action of clay in slowing the decomposition of organic matter in soil is well known, and it seems likely that this protective action can be extended to living organisms. Marshall (1964, 1975) showed that clay can enhance the survival of bacteria exposed to adverse conditions: thus more Rhizobium trifolii survived oven-drying in the presence of illite or montmorillonite than in the absence of these clays. On the other hand, Sorensen (1975) incubated labeled cellulose in a range of soils for 2 years and found that the biomass, as measured by the fumigation technique, was not directly related to the inorganic colloid contents of his soils. Clearly, clay content is not the only thing to influence the survival of organisms in soil—other factors, including organic matter content, temperature, freezing and thawing, drying and wetting, and development of anaerobicity will be involved. Much of the literature on the survival of individual species in soil, particularly the very extensive literature on the survival of plant pathogens (see Sussman, 1965), is relevant to the survival of microbial biomass in soil, although not reviewed here.

TABLE 3 Formation and Decline of Microbial Biomass in Soil

Substrate Added	Incubation Conditions	Soil	Period of incubation (yrs)	Labeled Substrate C Remaining in Soil (%)	C in Biomass (as % originally added substrate C[a])
^{14}C-labeled glucose[b]	Laboratory (25°C)	Silty clay loam	0.14	29	11.1
^{14}C-labeled glucose[c]	Field	Clay	0.25	39	19.8
^{14}C-, ^{15}N-labeled medic[d]	Laboratory (25°C)	Clay	0.09	49	9.9
			0.17	45	11.0
			0.26	45	10.0
			0.36	42	9.8
^{14}C-, ^{15}N-labeled medic[e]	Field	Sand	0.15	31	4.7
			1	23	1.7
			2	20	1.9
			4	17	0.9
^{14}C-, ^{15}N-labeled medic[e]	Field	Clay	0.15	42	7.4
			1	21	3.3
			2	19	3.3
			4	15	1.7
^{14}C-labeled ryegrass[f]	Field	Silty clay loam	1	32	7.0
			2	26	4.4
			3	22	3.1
			4	19	2.6
^{14}C-labeled straw[g]	Field	Sand	12	14	0.5

[a]Using $k_c = 0.45$.
[b]Jenkinson and Powlson (1976a).
[c]Shields et al. (1974).
[d]Amato and Ladd (1980).
[e]Ladd, Oades, and Amato (unpublished).
[f]Jenkinson and Rayner (1977).
[g]Sorensen (1977).

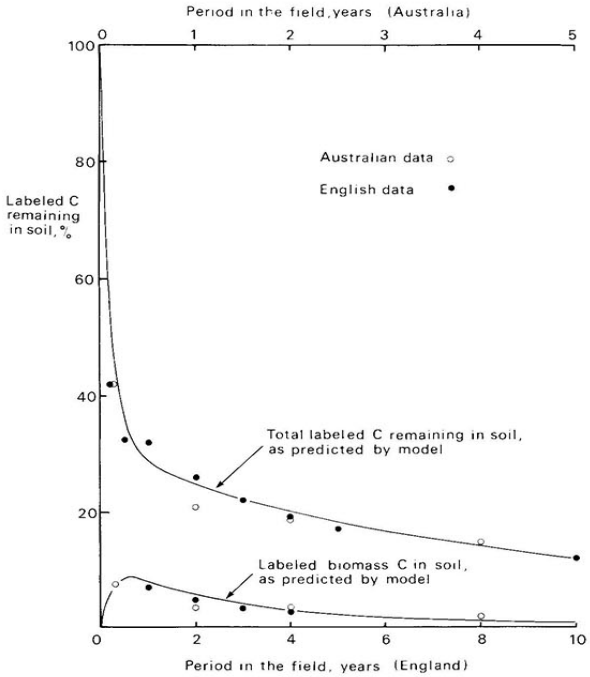


FIG. 5 Formation and decline of microbial biomass in the field under different climatic conditions. The Australian data were obtained with labeled medic decomposing in Northfield clay (Source: Ladd et al., 1981); the English with labeled ryegrass decomposing in Rothamsted soil (Source: Jenkinson and Rayner, 1977). The curves are as predicted by the model set out in Fig. 4.

D. Turnover Time of the Soil Biomass

By fitting the model of Sec. VII. A to the data in Table 3 the turnover time T, yield coefficient f_b, and decay constant r_b of the biomass can be estimated.

Two sets of data were used for the particular fit illustrated in Fig. 5, one from a field experiment on the formation of biomass during the decay of labeled medic in a South Australian clay soil (Ladd et al., 1981) and the other from a similar experiment on the decomposition of labeled ryegrass in England (Jenkinson and Rayner, 1977). The soils were chosen to be of similar pH and texture, so as to minimize soil, as distinct from climatic, differences. The two sets of results could be roughly superimposed by assuming that decomposition was twice as fast in the warmer Australian environment. The model was fitted to the combined data by adjusting the five parameters f_b, f_m, r_p, r_b, and r_m. The values the curves in Fig. 5 were:

f_b (= yield coefficient) = 0.1 in England and Australia

f_m = 0.22 in England and Australia

r_p = 4.5 in England, 9.0 in Australia

r_b = 0.4 in England, 0.8 in Australia

r_m = 0.1 in England, 0.2 in Australia

The agreement between the measured values and the predicted curves is not exact, but the accuracy of the data hardly warrants closer fitting, either by further adjusting the model parameters or by fine adjustment of the 2-to-1 relationship between the English and the Australian time scales. The corresponding value for the turnover time T (= $1/r_b$) in England is 2.5 yr (Australia 1.25 yr) and for the doubling time t_D (= T ln 2) of the population 1.7 yr (Australia 0.9 yr).

These turnover times are 3 to 4 orders of magnitude greater than those of organisms growing under optimal laboratory conditions. They are derived from measurements made long after the addition of fresh plant material and refer to a "stabilized" biomass, not to the active population present shortly after the addition. The calculations are based on a homogeneous single-compartment model that takes no account of the spread of turnover times that occur even in this "stabilized" biomass. In reality, some sections of the biomass will have turnover times less and some greater than the calculated value.

As with biomass, stabilized microbial metabolites should not be lumped into one compartment, at least two metabolite compartments being necessary to match present field data (Jenkinson and Rayner, 1977). However, one of these compartments had such a long turnover time, nearly 3000 yrs, that it can be neglected in the present context. Likewise, incoming plant material should not be put into a single compartment; a better fit to the experimental data over the first year could have been obtained had this not been done.

The fitted values for r_b, f_b, and f_m can be used to calculate B, the steady state biomass, if the annual input of plant carbon into the soil (A) is known (or A, if B is known). For the unmanured plot of the Continuous Wheat Experiment at Rothamsted, A is 1200 kg C ha^{-1} yr^{-1} (Table 4, Sec. VII). Assuming that the ryegrass data in Fig. 5 can be applied to the real field situation, where the input consists mainly of wheat roots and stubble, then the value of B calculated from the equation

$$A = \frac{r_b}{f_b}(1 - f_b - f_m)B$$

is 440 kg C ha^{-1}, of the same order as the measured value, 570 kg (Table 4). This rough agreement suggests that experiments on the decomposition of labeled substrates, such as those listed in Table 3, can give information relevant to the field situation.

E. Maintenance Energy

There is considerable evidence (Mallette, 1963: Marr et al., 1963; Pirt, 1975) from experiments on the growth of organisms in vitro that actively growing bacterial cells require a certain amount of energy per unit time, known as the maintenance energy, that is independent of the rate of growth (i.e., the production of new cells per unit time). This energy is required to maintain cell integrity by replacing unstable cell constituents, or constituents consumed, degraded or leaked during metabolism. It is also needed for cell motility and to maintain concentration gradients between the cell and its exterior. It is given by aB, where a is the "specific maintenance rate" (Marr et al., 1963) in yr^{-1}, defined as the fraction of the biomass replaced through maintenance each year. The term maintenance coefficient (m) is also used (Pirt, 1966), m being the amount of substrate needed for maintenance per unit biomass per unit time. Thus

$$aB = f_b mB$$

where f_b is the true yield coefficient.

It should be noted that, for the model set out in Sec. VII. A, the turnover time T is the same however the input of substrate is partitioned between material for cell division and material for maintenance. This is because the fraction (f_b) of the incoming substrate converted to biomass C is assumed to be the same whether used in maintenance or in producing new cells. In contrast, the relationship

$$t_D = T \ln 2$$

where t_D is the doubling time of the population, does not hold when some of the incoming substrate is diverted to maintenance.

The maintenance energy requirement of the soil population was first examined by Babiuk and Paul (1970) and later by Gray and Williams (1971), Shields et al. (1973), Hunt (1977), Barber and Lynch (1977), and Paul and van Veen (1978). All agree that specific maintenance rates must, at most, be very much smaller than those observed for organisms grown in vitro. This can be illustrated from the data in Table 4. If all the incoming substrate is used for maintenance and none for cell division, then the maximum amount of cell substance that can be replenished in unit time is $r_D f_b P$ ($= f_b A$), using the terminology of the preceding section. Under these conditions

$$aB = f_b A$$

For the unmanured Rothamsted soil carrying wheat B = 570 kg C ha^{-1}, A = 1200 kg C ha^{-1}, and $f_b = 0.10$, giving a maximum value of 0.21 yr^{-1} for a. This corresponds to a value of 0.000024 hr^{-1}, 3 orders of magnitude smaller than the value of 0.041 hr^{-1} calculated from Pirt's (1966) results for Aerobacter cloacae growing aerobically on glucose in a chemostat at 37°C. Marr et al. (1963) found the specific maintenance rate for Escherichia coli growing at 30°C to be 0.028 hr^{-1}.

The long turnover time of the soil biomass strongly suggests that few organisms are in active growth at any one time. The maintenance energy concept may not be applicable to resting organisms, cysts, spores, etc. which may rely on their endocellular reserves for respiration rather than exocellular substrates. Pirt (1966) pointed out that maintenance energy is associated with the growing state and that the maintenance energy requirement of growing cells is likely to be greater than that of organisms in a "stationary phase." As yet, there is no direct evidence for the consumption of substrate to maintain the largely resting population found in soils under natural conditions.

Soils contain small concentrations of free sugars (Lowe, 1978) and free amino acids (Kowalenko, 1978), and it is possible that much of these (and other substrates present in low concentrations) are used for maintenance rather than for the production of new cells.

VIII. CONCLUSIONS

The biomass has a double role in soil: as the agent of transformation through which pass all the natural organic materials that enter the soil, and also as a small, but labile, reservoir for N, P, and S. This is illustrated in Table 4, in which biomass data have been brought together for one

TABLE 4 The Microbial Biomass in an Unmanured Soil[a] under Continuous Wheat

Weight of soil[b]	2200	tonnes ha^{-1}
Organic matter in soil[b]	26	tonnes C ha^{-1}
N in soil[b]	2.7	tonnes N ha^{-1}
Annual input of organic matter[c]	1.2	tonnes C ha^{-1} yr^{-1}
Gross turnover time of soil organic C[c]	22	yrs
Radiocarbon age of soil organic C (1944 sample)[c]	1310	yrs
Number of "spherical" organisms[d]	1100	million g^{-1}
Volume of "spherical" organisms[d]	0.71	mm^3 g^{-1}
Number of hyphae[d]	7	million g^{-1}
Length of hyphae[d]	140	m g^{-1}
Volume of hyphae[d]	0.97	mm^3 g^{-1}
Number of bacteria and actinomycetes (plate count)[e]	44	million g^{-1}
Number of bacteria and actinomycetes (direct count)[e]	1600	million g^{-1}
Fraction of pore space occupied by organisms[f]	0.35	%
Microbial biomass from biovolume[d]	220	μg C g^{-1}
Microbial biomass from flush[d]	220	μg C g^{-1}
Microbial biomass from flush[b]	570	kg C ha^{-1}
ATP content of soil[g]	1.22	μg ATP g^{-1}
Turnover time of biomass C[h]	2.5	yrs
Maximal value for specific maintenance rate[h]	0.21	yr^{-1}
N in biomass[i]	95	kg N ha^{-1}
Flux of N through biomass[j]	38	kg N ha^{-1} yr^{-1}
P in biomass[k]	11	kg P ha^{-1}
Flux of P through biomass	5	kg P ha^{-1} yr^{-1}

[a]Broadbalk plot 03, from Rothamsted, sampled to a depth of 23 cm. All results are on an oven-dry basis.
[b]Jenkinson and Powlson (1976b).
[c]Jenkinson and Rayner (1977).
[d]Jenkinson et al. (1976).
[e]Skinner et al. (1952); mean of 7 measurements made over a period of 6 months.
[f]Assuming a true soil density of 2.5 g cm^{-3}.
[g]Jenkinson et al. (1979).
[h]See text.
[i]Assuming a C/N ratio of 6 for the microbial biomass.
[j]N in biomass : turnover time of biomass C.
[k]Assuming a C/P ratio of 50.

particular soil from Rothamsted. The soil is from the unmanured plot of the Broadbalk Continuous Wheat Experiment and has carried wheat almost every year since 1843. There have been no measurable changes in the organic C and N contents of soil from this plot since at least 1881, so that the soil can be assumed to have reached steady-state conditions, with the annual input of organic matter from the wheat crop just balanced by the annual loss of CO_2 from the soil (Jenkinson and Rayner, 1977).

In Table 4, the C/N ratio of the biomass has been taken as 6, to give a flux of N through the biomass of 38 kg N ha^{-1} yr^{-1}. This is of the same order as the annual offtake of N in grain and straw from this plot (24 kg N ha^{-1} yr^{-1}; Jenkinson, 1977), and suggests that the biomass pool is capable of supplying a significant part of the N taken up by an unfertilized crop. The unmanured plot in Broadbalk is at a very low level of fertility and contains less biomass than more favored soils (Table 2), in which the flux of N through the biomass will presumably be greater.

The P content of soil organisms grown in vitro varies over a wide range (van Veen and Paul, 1979) and little is yet known about the C/P ratio of the soil biomass in situ, or of how this ratio differs between soils. Setting the C/P ratio provisionally at 50 gives a P flux of 4.6 kg P ha^{-1} yr^{-1} through the biomass, to be compared with an offtake in grain plus straw of about 5 kg P ha^{-1} yr^{-1}. In a grassland soil, with perhaps 4 times as much biomass C as in the unmanured Broadbalk plot, the P flux will presumably also be larger.

From the point of view of the soil chemist, the biomass is a small but labile fraction of the soil organic matter that makes a contribution to plant nutrition far greater than its size might suggest. From the point of view of the microbiologist, the picture of the soil biomass that is now emerging is of a huge, largely dormant population, with two unique features: an enormous richness of species and an ability to survive hard times. The first of these is an evolutionary response to the multiplicity of substrates and environmental conditions, both on the micro and the macro scale, that occur in soil (Szabó, 1974; Russell, 1973; Stotzky, 1972; Alexander, 1971). Within wide limits there will always be a species (or more usually a suite of species) capable of using a given naturally occurring substrate in a given environment. The second arises because most of the soil population must wait passively for food, none but a few of the larger organisms being able to take active steps to search it out. While waiting, the organisms must be able to survive the soil environment, not many being large and active enough to move away from places that have become too dry, too hot, or too cold. A large biomass and a low metabolic rate are advantageous to a species living under these conditions. The physiological and biochemical changes necessary to achieve a low metabolic rate are considered elsewhere (Gray and Williams, 1971; Brock, 1971; Gray, 1976; Dawes, 1976; Boylen and Mulks, 1978).

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